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Application of phase contrast microscopy to the study of marine micro-biota

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ABSTRACT

We present the observation of the activity of artemia, one of the popular marine micro-biota species, in free space by the application of Fourier optics imaging technique. The Fourier optic imaging system is consisted by a collimated laser beam source, a Fourier spatial filter, an non-coherent IR source, and a CCD imaging system. By recording the images of Artemia's motion in real life, we are able to study the fundamental patterns of artemia motion mechanism, and the response of the motion pattern to the variation of its environment. Characteristic patterns of artemia's motion, such as linear motion, spiral motion, and mating collision are observed. It is shown that the increasing of the environment temperature driving the motion of artemia's moving faster and more frequently, and still saves alive even at the environment temperature up to 38 C.

Keywords: Artemia, brine shrimp, Fourier optics imaging

1.1. Introduction

The life form of micro-biota is closely linked with the oceanic life. Algae and bacteria are primary food producer, and micro-biota are their predators. Micro-biota in turn is preyed by fishes, thus the status of micro-biota's living is known as an indicator of the marine life. It is important for us to know what the environmental perturbation and pollution might cause damage to the micro-biota living system, and the threatening to the ecological status of marine life. Since, most of the micro-biota is transparent, low contrast relative to their environment, and microscopically small; range in size from tens of micrometers to few millimeters, thus causing challenge in observing their living behavior in free space. J.R. Strickler, and J. S. Hwang had developed a Fourier optic imaging system which used a spatial filter to observe a phase object in long working distance(1-5), and showed that Fourier imaging technique is an effective approach to study the life motion of the tiny, transparent marine objects.

In this paper, we present the application of Fourier transform imaging system to study the activity of the Artemia in free space. Artemia known as the brine shrimp is in the phylum Arthropoda, class Crustacea. Artemia are zooplankton like Copepods and Daphnia, which are the primitive food source for the early stages of many fish and crustacean larvae (6). Since artemia is easily produced in the lab, and its response to the variation of environmental factors, such as temperature, light illumination, PH value and oxygen contain in the water can be repeatedly controlled in the experiments, artemia is an ideal sample of studying the micro-biota in marine life. In addition, the Fourier transform images is able to reveal more detailed features of artemia behavior in real life than by conventional imaging technique,

1.2. Experimental setup

The schematics of the Fourier optic system is shown in Fig 1, a collimated laser beam of plane-wave and uniform intensity is illuminating the objects S_o which is optically transparent. The diffracted light from the object then pass through a Fourier transform lens L_1, and is focused on the spatial filter. An high pass spatial frequency filter and the lens L_2 are used to inversely Fourier transform the diffracted pattern of the observed object and to image by the CCD.
The artemia life cycle begin by the hatching of dormant cysts, which are encased embryos that are metabolically inactive. The cysts can remain dormant for many years as long as they are kept dry and oxygen free. When the cysts are placed back into salt water they are re-hydrated and resume their development. The conditions for hatching artemia is :25 degrees C, salinity - 5 ppt (1.030 density), heavy continuous aeration, light - 2000 lux constant illumination, pH around 8. Good circulation is essential to keep the cysts in suspension. After 15 to 20 hours cyst bursts and the embryo leaves the shell. For the first few hours, the embryo hangs beneath the cyst shell, still enclosed in a hatching membrane. This is called the Umbrella stage, during this stage the nauplius completes its development and emerges as a free swimming nauplii. Approximately 12 hours after hatch they molt into the second larval stage and they start filter feeding on articles of various

In *Optical Sensing, Imaging, and Manipulation for Biological and Biomedical Applications*, Robert R. Alfano, Ping-Pei Ho, Arthur E. T. Chiou, Editors, Proceedings of SPIE Vol. 4082 (2000) • 0277-786X/00/$15.00

microalgae, bacteria, and detritus. The nauplii will grow and progress through 15 molts before reaching adulthood in at least a week. Since artemia are non-selective filter feeders, a wide range of food can be used, such as bread yeast, wheat flour, soybean powder, fish meal, and egg yolk.

For the observation of artemia motion in real life, we filter the adult artemia in valuable numbers out from the cysts in the hatching container, then put into the observation vessel which has the dimension of 12cmx5cmx15cm and is made by glass . The Fourier optic system is aligned with the collimated laser beam at 660 nm first , after a clear Fourier transform image is achieved, we cut off the incident of the alignment laser beam, and using an IR non-coherent light source to illuminate the objects in the vessel. In order to eliminate the imaging noise due to the diffraction caused by micro-alga or sediment in the water, instead of using a coherent laser source for illumination, we introduce an IR non-coherent collimated light source illuminating aside the vessel. Figuer 2 shows the improvement of the Fourier transform image quality by using the IR non-coherent light source.

1.3. Results & Discussion

We study the motion patterns of artemia by the Fourier transform images recorded by the CCD and the PC based digital image acquisition system. Figure 3 shows a series of images of the continuos motion of the artemia . The pictures show that the basic driving mechanism of artemia forward motion is by swimming of the pair of its antenne which is also the sensing element to the variation of the environment. Figure 4 shows the most commonly seen spiral tracing of artemia motion, and shows that the direction of artemia's tail, named as postmaxillar region, is along its spiral trace, and that is the evidence of proving that the tail of artemia has the function of controlling its direction of motion, and the balancing of the body in swimming .

Since that mating behavior is important not only to the generation of artemia ecology of life but other marine species as well. Figure 5 shows the mating behavior of adult artemia. The mating collision time is measured by the recording time between the CCD images, and is estimated about the mill-seconds order. The observation shows that the probability of success second encounter is smaller than 2%. Since not all of the encountering are mating collision, certain ration of encountering are escaping that provides the evidence of the behavior of selection the mating partner.

Temperature is the key factor of effecting the marine life physically or mechanically. In our experiments of observing the response of artemia motion patterns by locally heating of its environment using high power IR irradiation. The temperature of the sea water can be heated from 18 ^{0}C to 38 ^{0}C at a temperature ramping of 0.5 C/minute. The results shows that there is not significant changes of the fundamental patterns of artemia's motion. However, it is found that the speed of motion does increase with the increasing of the temperature of its environment. In principle, the higher the temperature, the smaller the viscosity of the sea water is, and thus might cause artemia moving faster in the laminar flow. It might suggest that the effect of temperature to artemia motion behavior is mostly due to the change of mechanical property of the sea water. The artemia saves alive even at the environment temperature up to 38 C. This revealed that the artemia has wide tolerance to the variation of the temperature.

It is known that the pair of the antenne of artemia are important elements of sensing the perturbation of its environment. Marine biologists are interested of knowing the answer of how long is the limited distance of artemia's perception. Since the limitation of artemia perception might be closely related with its ecological domain size, and the interaction between the artemia. From the observation of artemia encountering, it is shown that the perception distance is about two to three times the length of artemia body.

1.4. Conclusion

As a conclusions, we shows the improvement of the Fourier transform microscopy technique by using a non-coherent IR source, and the application of this system in observing the behavior of artemia in real life. The Fundamental mechanism of the artemia's driving motion has been concluded as the swimming mechanism in a laminar flow, and the pair of antenna are the swimming arms. The mating encountering time is estimated in the order of mini-seconds, and the selectivity of mating partner has been observed. It is suggested that the effect of temperature to artemia is due to the change of the mechanical property, the reasonable answer might be the change of viscosity to effect the motion of artemia mechanically. Although, a 532 nm laser beam has been used to study the visual response of artemia to the illumination condition of the environment, particularly the spectrum response, there is no conclusion due to the lack of observation data, and further study is needed to conclude the facts.

ACKNOWLEDGEMENTS

The authors would like to thank professor J. R. Strickler, or his generous help to improve the optical system, and graduate student of professor J. S. Hwang for the help in hatching the artemia cysts. We acknowledge financial support by the Asia Pacific Research Foundation and National Science Council.

REFERENCES

1. Trager G. C., Hwang J. -S., and Strickler J. R. (1990). Barnacle suspension-feeding in variable flow. Marine Biology. Vol.105: 117-127.
2. Hwang J. -S., Turner J. T.,Costello J. H., Coughlin D. J., and Strickler J. R. (1993). A cinematographic comparison of behavior by the calanoid copepod Centropages hamatus: Tethered versus free-swimming animals. Journal of Experimental Marine Biology and Ecology. Vol. 167: 277-288.
3. Hwang J. -S.,Costello J. H., and Strickler J. R. (1994). Copepod grazing in a turbulent flow: elevated foraging behavior and habituation of escape responses. Journal of Plankton Research.Vol.16(5): 421-431.
4. Strickler J. R., and Hwang J. -S. (1998). Matched spatial filters in long working distance microscopy of phase objects. In Wu J. -L., Hwang P. -P., Wong G., Kim H., Cheng P. -C. (eds.) Focus on Multidimensional Microscopy. World Scientific Publishing Co., Singapore (In press). (Chapter in the textbook).
5. Bardach, J.E., J.H. Ryther and W.O. McLarney. 1972. Aquaculture: the farming and husbandry of freshwater and marine organisms. Wiley Interscience, New York, 868 pp.
6. Robert A. Browne, Patrick Sorgeloos, Clive N. A. Trotman, Artemia Biology, CRC press, p.166, 1991.

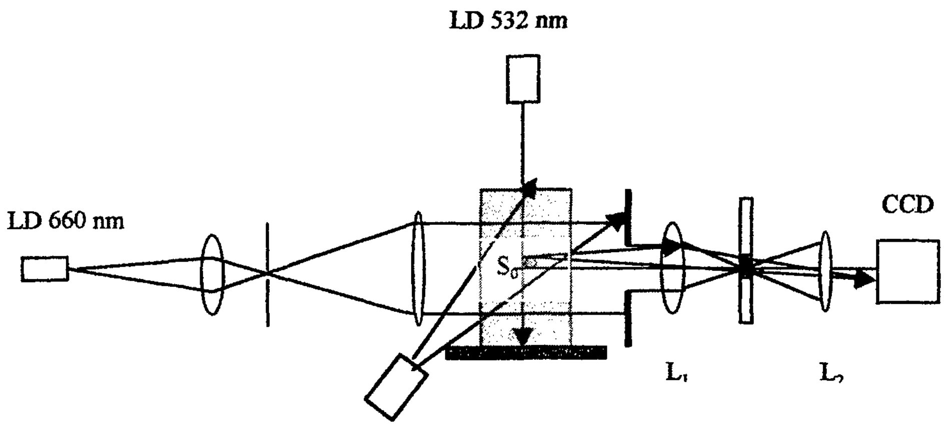
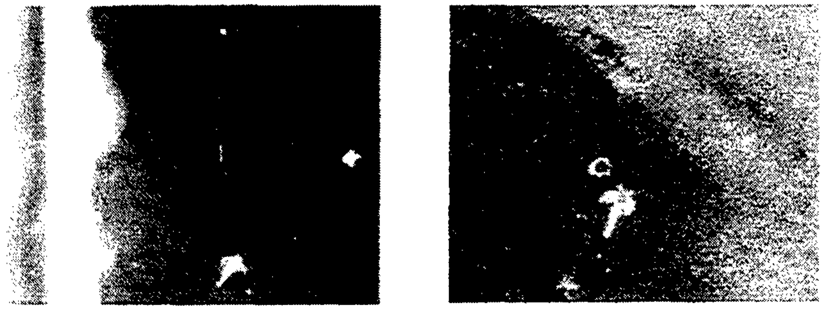


Fig 1. The schematics of the Fourier optic system as shown, a collimated laser beam of plane-wave and uniform intensity is illuminating the objects S_0 which is optically transparent. The diffracted light from the object then pass through a Fourier transform lens L_1, and is focused on the spatial filter. An high pass spatial filter and the lens L_2 are used to inversely Fourier transform the diffracted pattern of the observed object and to image by the CCD.



Figuer 2. Left hand side is the Fourier transform image by using non-coherent IR light source, and the right hand side is taken by the coherent laser source. It shows the improvement of the Fourier transform image quality by using the IR non-coherent light source.

Figure 3 shows a series of images of the continuos motion of the artemia . It shows that the basic driving mechanism of artemia forward motion is by swimming of the pair of its antenne.

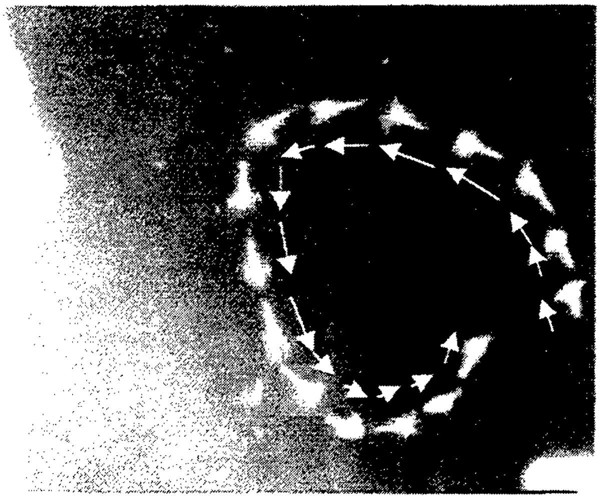


Figure 4. The most commonly seen spiral tracing of artemia motion. The artemia's tail, named as postmaxillar region, is to control its direction, and the balance in swimming .

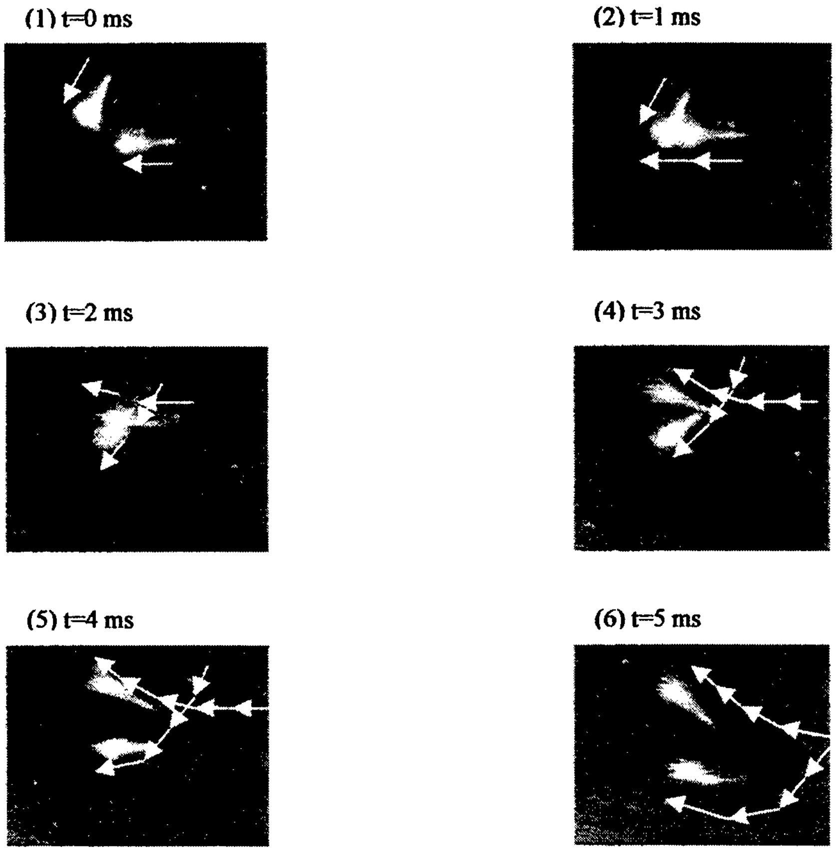


Figure 5. The series of photos show the mating behavior of adult artemia. The mating collision time is estimated about the order of mill-second..